

Investigation of mercury exposure in Maine's Mink and River Otter

(BRI 2002-10)

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Submitted to:

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Abstract

Anthropogenic releases of mercury into the environment for the past several decades have collected in aquatic ecosystems. The impact of this mercury build-up is of concern to regulators and policy makers. Maine and much of New England are especially at high risk because of local and regional emission sources, prevailing wind patterns, and certain hydrological and biogeochemical features. This study helps establish an exposure profile for mercury in mink and river otter populations in Maine. Although a total of 26 otter and 47 mink carcasses have been collected, parametric statistical analysis of covariables is not yet possible. Mercury levels do tend to be greater in mink vs. otter, interior vs. coastal populations, and females vs. males. Respective mean mercury levels in otter and mink fur, 19.6 and 21.8 ppm, were near concentrations considered to have adverse effects in other studies. The proportion of sampled populations exceeding 20 ppm in the fur was 61% for otter and 47% for mink. Mink fur Hg levels ranged up to 68.5 ppm. Brain and liver Hg levels were well below published lethal levels. The strong relationship among brain, liver, and fur Hg levels indicates great flexibility in using one compartment for determining mercury exposure. Otter and mink mercury levels from western and northern Maine indicate greatest risk. Continued collection of carcasses through our established trapper network will increase sample size and geographic scope. Soon, we will have a suitable mercury exposure profile that can be used to model a wildlife criterion value protective of Maine's mink and river otter population.

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INTRODUCTION

Mercury and other aquatic-based persistent bioaccumulative toxins are prevalent in Maine's freshwater and marine environments (Maine DEP 1998). Methylmercury (MeHg) availability to fish and wildlife varies geographically and is strongly influenced by hydrology and biogeochemical factors (Evers and Reaman 1998, Evers et al. 1998b). To interpret exposure levels in wildlife established benchmarks are needed. Therefore, standardized sampling of target biosentinels provides a method for making informed comparisons and definitive interpretations, thereby helping to assess risks to wildlife and allow landscape-level extrapolations of the hazards.

The mink and the river otter are both widely distributed in New England and Maine. Both species have diets that include fish and crayfish, although mink are known generalists. Because of their high metabolism and piscivorous diet, both mink and river otters are highly susceptible to elevated levels of environmental MeHg. Brain and liver samples are an effective way of showing total Hg levels that occur in these animals. Fur indicates the body burden or total accumulated Hg during the animal's life.

As in 2000, our objective in 2001 was to obtain determine Maine's mercury exposure profile for mink (*Mustela vison*) and river otter (*Lutra canadensis*) based on tissues from carcasses provided by trappers.

Context

Lab-based, dose-response studies of mink (Wobeser et al. 1976) and otter (O'Connor and Neilson 1980) have shown that terminal total Hg concentrations occur at 25 parts per million wet weight (ppm, ww) in the liver and kidney. Thompson (1996) estimated that 30 ppm (ww) of total Hg in the liver or kidney is at least sublethal and potentially lethal. He also reported that dietary MeHg concentrations of 2 to 6 ppm (ww) were "sufficient to cause mercury intoxication."

Although fish total Hg levels over 2 ppm occur in Maine, they are relatively rare. However, fish total Hg levels greater than 1 ppm are common. Evers and Reaman (1998) found fillets from land-locked salmon (*Salmo salar*) in Pierce Pond (1.06 ppm, ww for a 53 cm fish), yellow perch (*Perca flavescens*) in Mooselookmeguntic (1.11 ppm, ww for a 34 cm fish), and Yellow Perch in Flagstaff (1.26 ppm, ww for a 29 cm fish) exceeded these levels. They also found fillet Hg levels to significantly increase as fish size (indexed by length x weight) increased for land-locked salmon, smallmouth bass (*Micropterus dolomieu*), and yellow perch. Nearly all Hg in fish is MeHg (Wiener and Spry 1996).

As evidenced by empirical studies conducted by BioDiversity Research Institute (BRI) in Maine and comparisons with other studies (Table 1 and 2), mink and river otter are likely exposed to sufficient quantities of dietary Hg to cause sublethal impacts. Evers et al. (1998a) found Common Loon (*Gavia immer*) Hg levels have an increasing west to east trend in North America. Mean juvenile loon blood mercury levels from Maine were 4.5x higher than Alaska and 2x higher than the upper Great Lakes and Ontario. On several of Maine's reservoirs (e.g., Flagstaff Lake), juvenile loon blood Hg levels were up to 10x higher than Great Lakes sites.

Table 1. Concentrations of total Hg (ppm,ww) in river otter from various study sites. All values in parentheses are ranges and single values are means.

Site	Sample Size	Tissue	Muscle	Brain	Liver	Kidney	Fur*	Source
<i>Ireland</i>	32	-	-	-	(0.15-17.03)	-	-	Mason 1993
<i>Denmark</i>	69	-	-	-	(0.03-12.37)	-	-	Mason 1992
<i>Britain</i>	7	-	-	-	(0.17-4.33)	(0.08-2.02)	-	Mason 1988
<i>NY</i>	-	-	-	-	2.35	-	-	Mayack pers. com.
<i>ON 1</i>	1**	36**	30**	96**	58**	47**		Wren 1985
<i>ON 2</i>	-	0.89	-	2.97	1.05	-		Wren 1980
<i>ON 3</i>	-	-	-	(1.0-3.5)	-	-		Wren 1988
<i>ON 4</i>	130	-	2.0	6.7	-	13.8		Mierle 2000
<i>WI</i>	49	1.44	0.74	3.34	8.47	6.47		Sheffy 1982
<i>VT</i>	21	-	-	-	-	13.58 (4.91-46.5)		BRI Unpub. Data 2002
<i>ME</i>	27	-	0.44 (0.08-1.03)	1.89 (0.24-4.74)	-	20.25 (1.1-37.6)		BRI Unpub. Data 2002

* Fresh weight

** *Based on one individual from the English-Wabigoon River system that contained a chlor-alkali plant recently operating; this otter was found dead due to mercury exposure.*

Because the prey base is similar to loons, we expected body burdens in Maine to be greater than those in other areas of the United States for river otter (Table 1) and mink (Table 2). Much of the literature is based on liver total Hg levels. However, because much of the Hg in the liver is demethylated (Scheuhamer et al. 1998) the available toxicity of methylmercury is best measured in the brain, fur, or muscle tissue.

Table 2. Concentrations of total Hg (ppm,ww) in mink from various study sites. All values in parentheses are ranges and single values are means.

Site	Sample Size	Tissue	Muscle	Brain	Liver	Kidney	Fur*	Source
WI	39		1.26	0.46	2.08	2.33	7.61	Sheffy 1982
CT	8		-	-	(1.1-8.47)	-	-	Major 1991
MA	4		-	-	(0.008-1.92)	-	-	Major 1991
NY	-		-	-	2.35	-	-	Mayack pers. com.
OH	-		-	-	0.135	-	-	Lynch 1973
PQ 1	-		1.87	0.83	9.23			Desai-Greenway 1976
PQ 2	-		2.4 (0.41-6.2)	-	8.34 (2.21-20.0)	-	-	Langis 1999
SK	1**		-	-	58.2	31.9	-	Wobeser 1976
ME	47		-	0.61 (0.13-2.55)	2.05 (0.27-8.03)	-	21.76 (1.78-68.5)	BRI Unpub. Data 2002

* Fresh weight

** Mink found in wild alive but later died due to mercury exposure

STUDY AREA & METHODS

Study Area

Previous mercury-based studies in Maine provided information on “hotspots” (Welch 1994) (Evers et al. 1998a), aquatic habitats prone to enhanced methylmercury availability (Evers and Reaman 1998), and species most at risk (Evers et al. 1998b).

We identified three focal areas, although carcasses were collected from areas statewide: (1) Flagstaff Lake, the North Branch of the Dead River and its watershed including Chain-of-Ponds, and the Dead River outflow from the Flagstaff dam have some of the highest levels of biotic mercury in the country. (2) Seboomook and Canada Falls Lakes and neighboring areas have had reports of mink extirpations. This watershed is at high elevation with known high mercury endpoints. (3) St. John’s river area around Daaquam was chosen because of elevated mercury levels found from 2000 opportunistic sampling. Other animals were taken from areas around the state. For actual location of carcasses collected, see appendices 1 and 2.

Sample collection and processing

We collected 26 river otter (8 from 2000 and 18 from 2001) and 47 mink (24 from 2000 and 23 from 2001) carcasses from licensed fur trappers during the 2000 and 2001 trapping seasons. Carcasses were stored on-site in freezers and were properly labeled (containing waterbody name and town of the trapping site). These carcasses were regularly retrieved by BRI staff. Brain, femoral muscle, liver tissue, and the lower jaw

were removed using stainless-steel instruments and placed into sterilized I-CHEM® jars. The lower jaw was then archived in a freezer so a canine tooth can later be removed to accurately age the animals. Fur was taken from the foot of the animal using stainless-steel instruments then cleaned and placed into sealed envelopes. The tissues, once harvested, were refrozen until they were sent to the lab. The tissue samples were harvested at the University of Southern Maine's Biology lab using techniques according to Tufts University Animal Wildlife clinic protocols (M. Pokras, pers. com.).

Fur, brain and liver tissues were analyzed for total mercury using Cold Vapor Atomic Absorption (CVAA) methods. Laboratory analysis was conducted by Texas A&M Trace Element Research Lab (TERL), College Station, Texas and Maine Environmental Lab (MEL), Yarmouth, Maine. Femoral muscle tissue were archived for future analysis. TERL and MEL has conducted BRI's mercury analysis for bird tissues (blood, feathers, and eggs), fish, and crayfish for the past three years. Mercury level results are given as fresh weight for fur and wet weight for liver and brain. Methylmercury levels were not analyzed.

Contacts for retrieving carcasses

The principal investigator discussed logistics of carcass retrieval with the following trappers: Dave MacNeill of Millinocket, Dan Kusnierz of Old Town, Bobby Cercena of Eustis, Jerry Le Beau of North Anson, Lindsay Seeley of Orrington, Jim Carter of Ashland, Oscar Cronk of Wiscasset, and Bruce Connery of Acadia National Park. In 2000, he also met trappers in the Boothbay area during a trapper safety course sponsored by Maine Inland Fisheries and Wildlife (where he received his trapping certificate # METS-025-00-006).

Methods for Live Trapping

We attempted live-captures of river otter and mink at latrine sites from September 25 to November 1, 2001. One-and-one-half inch soft catch foothold traps were set at entrances and exits of the latrine sites. We sectioned off the entrance and exits of the latrine sites and set the traps where the otter and mink are forced to step on them while traveling to or from the latrine. Also, traps were set on crossing paths that otter and mink use while traveling from one water-body to another. The number of traps varied at latrine sites from two to four depending on how many entrances and exits were present.

All of the traps were set on land, using a drop of otter or mink lure. The traps were equipped with four swivels and a spring to minimize trauma to the animal's foot. One swivel at the base of the trap allowed the trap to rotate 360°. The traps were anchored to a nearby root or a three-foot stake. The traps were always set so the animal could not reach the water or a tree to pry itself free and to avoid the potential risk of injury. We dug the traps into substrate so they would be flush with the ground. We used wax paper over the pan to keep the trigger debris free. The trap was then covered by pine needles and dirt to camouflage them. We checked the traps every morning so the animals were not in the traps for more than a night.

Once the otter or mink was captured we used a catchpole to safely control the animal while placing it in a holding box for transport back to our field station. We hand injected the animals using a mixture of Ketamine (2.5 mg/kg) and Medetomidine (0.025

mg/kg). We used Atipamezole (0.100 mg/kg) as an antiseden to the Medetomidine. The maximum time the animals were anesthetized was 45 minutes before given the antiseden.

RESULTS AND DISCUSSION

A. Carcass Collection

1. River Otter

We analyzed 18 fur, liver and brain samples in 2001. Fur Hg concentrations ranged between 1.14 ppm in otters from Round Pond, in Acadia National Park, to 32.0 ppm on Munsungan Pond (Table 3). Otter fur Hg levels indicates individuals from several sites are elevated (Table 1). Brain total Hg levels ranged from 0.23 to 1.03 ppm while liver total Hg levels ranged from 0.32 to 3.47 ppm (Table 3).

Wren (1985) showed that Ontario river otters with mean fur Hg levels of 47 ppm had on average 30 ppm and 96 ppm total Hg in the brain and liver respectively. Lethal levels are considered 30 ppm total Hg in the liver (Thompson 1996) and 19 ppm total Hg in the brain (Mierle et al. 2000). Although our fur Hg levels approach lethal levels, brain and liver Hg levels indicate lower than expected exposure.

Table 3. Concentrations of Total Hg levels (ppm, ww) in brain, liver, and fur* from river otters collected in Maine during 2000-01 trapping season.

Tissue	Year	Sample size	Mean	SD	Range
Brain Hg	2000	8	0.45	0.24	0.08 - 0.69
Liver Hg	2000	7	3.00	1.61	0.24 - 4.74
Fur* Hg	2000	8	21.60	11.28	4.99 - 33.7
Brain Hg	2001	18	0.44	0.18	0.23 - 1.03
Liver Hg	2001	18	1.53	1.01	0.32 - 3.47
Fur* Hg	2001	18	18.69	7.62	1.14 - 32.0
Total Brain Hg	2000-01	26	0.44	0.20	0.08 - 1.03
Total Liver Hg	2000-01	25	1.94	1.35	0.24 - 4.74
Total Fur* Hg	2000-01	26	19.58	8.60	1.14 - 33.7

*Fresh Weight

Fur Hg levels reflect the total body burden bioaccumulated over time, particularly for individuals with high exposure. Consequently the animal's age may be a confounding factor in interpreting fur Hg results. Mierle et al. (2000) found that Hg concentrations in fur changed with age. It increased during the first four years in Ontario otters, but then declined. However, fur Hg levels in the Ontario study did not exceed 15 ppm in known age otters, and it is likely the animals were able to demethylate their Hg body burden. In our study, several otters had relatively high fur Hg levels; therefore it is not clear if these animals would be able to demethylate their body burden. Blood Hg levels reflect recent dietary uptake and would help explain fur Hg concentrations.

2. Mink

We analyzed 23 fur, brain and liver samples in 2001. Mink fur Hg concentrations ranged from 1.78 ppm on Felts Brook near Orrington to 51.8 ppm on Red Pine Brook near Daaquam (Table 4). Mink brain and liver Hg ranged from 0.22 (brain) and 0.27 (liver) to 2.55 (brain) and 6.13 ppm (liver) from Ross Stream near Daaquam and St. Johns River respectively (Table 4).

Table 4. . Concentrations of Total Hg levels (ppm, ww) in brain, liver, and fur* from mink collected in Maine during 2000-01 trapping season.

Tissue	Tissue, Year	Sample size	Mean	SD	Range
Brain Hg	2000	24	0.63	0.46	0.13 - 2
Liver Hg	2000	24	2.46	1.92	0.49 - 8.03
Fur* Hg	2000	24	24.32	14.24	9.2 - 68.5
Brain Hg	2001	23	0.59	0.56	0.22 - 2.55
Liver Hg	2001	23	1.61	1.43	0.27 - 6.13
Fur* Hg	2001	23	19.09	13.53	1.78 - 51.8
Total Brain Hg	2000-01	47	0.61	0.51	0.13 - 2.55
Total Liver Hg	2000-01	47	2.05	1.73	0.27 - 8.03
Total Fur* Hg	2000-01	47	21.76	14	1.78 - 68.5

*Fresh Weight

B. Tissue Analysis

1. Liver

All liver samples were below the lethal levels of 25 ppm as reported by Wobeser et al. (1976); although extrapolating findings from controlled lab experiments to wild populations are difficult. Liver total Hg levels are best used for historical comparisons and our mean levels are generally similar to or less than those documented in other studies for otter and mink. Recent work has shown the percentage of MeHg in the liver reaches an upper limit and does not correlate with total Hg levels (D. Evans, pers. com.). Therefore, evaluating the impact of Hg toxicity using liver Hg levels is not recommended.

2. Brain

Because Hg is a neurotoxin and has the ability to cause deterioration of brain cells, this tissue is an important compartment for understanding the pharmacokinetics of Hg.

3. Fur

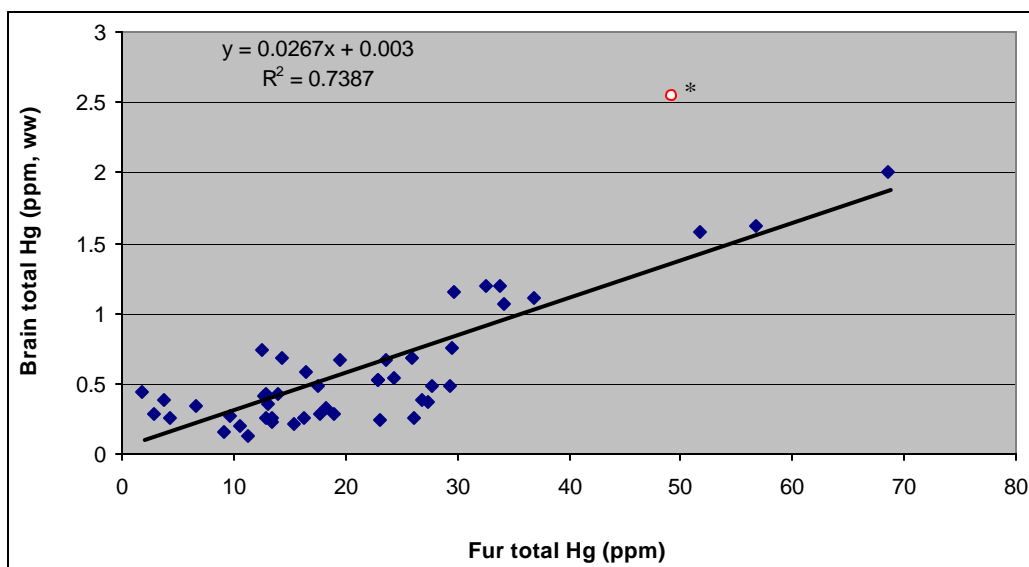
Measurement of Hg in the fur is an easily accessible compartment that is known to indicate chronic levels (Thompson 1996).

C. Relationship Between Tissues

1. Fur vs. Brain

The relationship between fur and brain Hg levels were highly significant for mink ($r^2 = 0.74$, $F = 124.4$, $df = 44$, and $p < 0.001$) (Figure 1a).

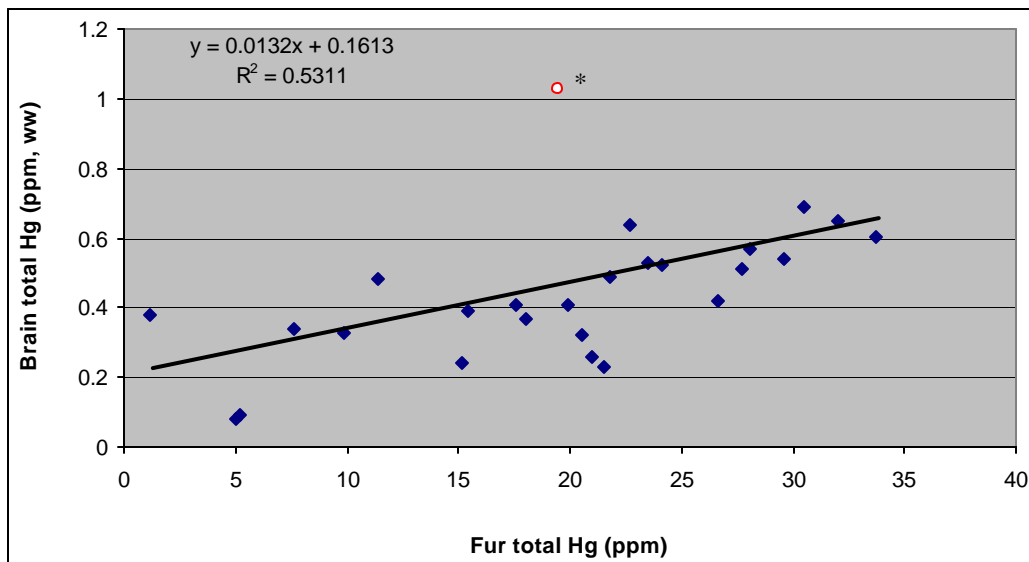
Figure 1a: Relationship between fur and brain Hg in Mink, Maine.



* This point was excluded from the regression (Figure 1a)

The relationship between fur and brain Hg levels were significant for river otter ($r^2 = 0.53$, $F = 26.1$, $df = 23$, $p < 0.0501$) (Figure 1b).

Figure 1b: Relationship between fur and brain Hg in River Otter, Maine.

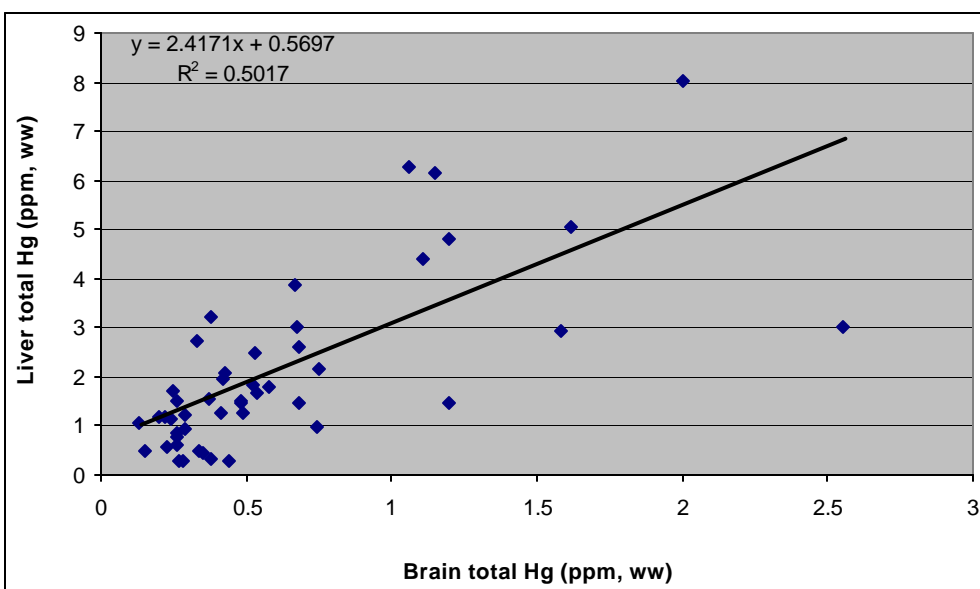


* This point was excluded from the regression (Figure 1b).

2. Brain vs. Liver

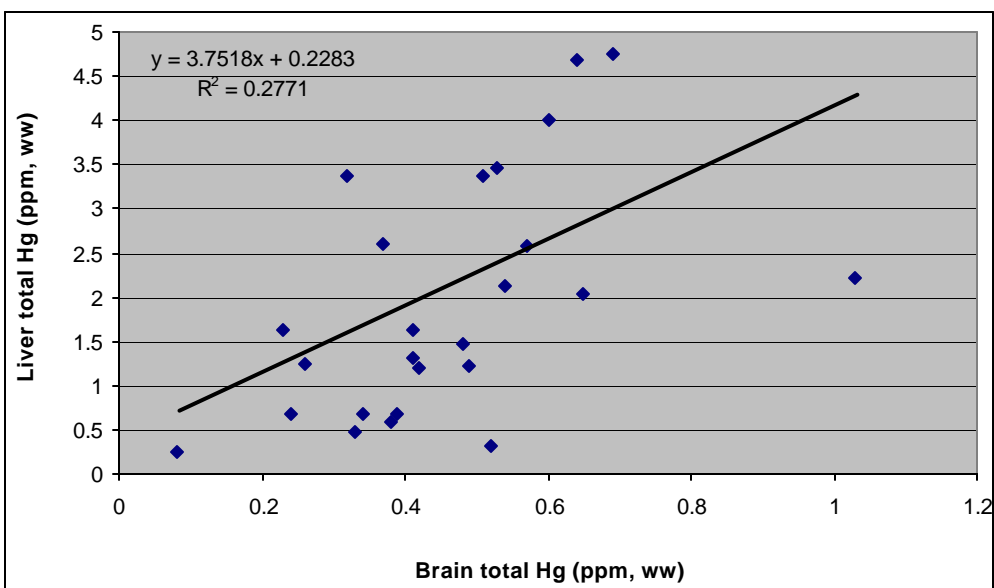
The relationship between brain and liver Hg levels were significant in mink ($r^2 = 0.50$, $F = 45.3$, $df = 45$, $p < 0.05$) (Figure 2a).

Figure 2a: Relationship between brain and liver Hg in Mink, Maine.



The relationship between brain and liver Hg levels were significant in river otter ($r^2 = 0.28$, $F = 8.81$, $df = 23$, $p < 0.05$) (Figure 2b).

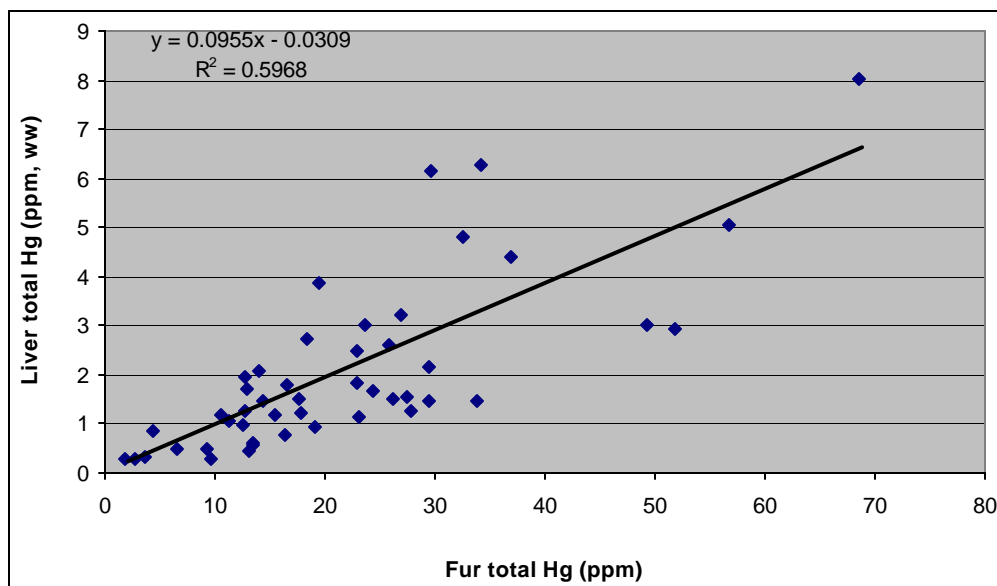
Figure 2b: Relationship between brain and liver Hg in River Otter, Maine.



3. Fur vs. Liver

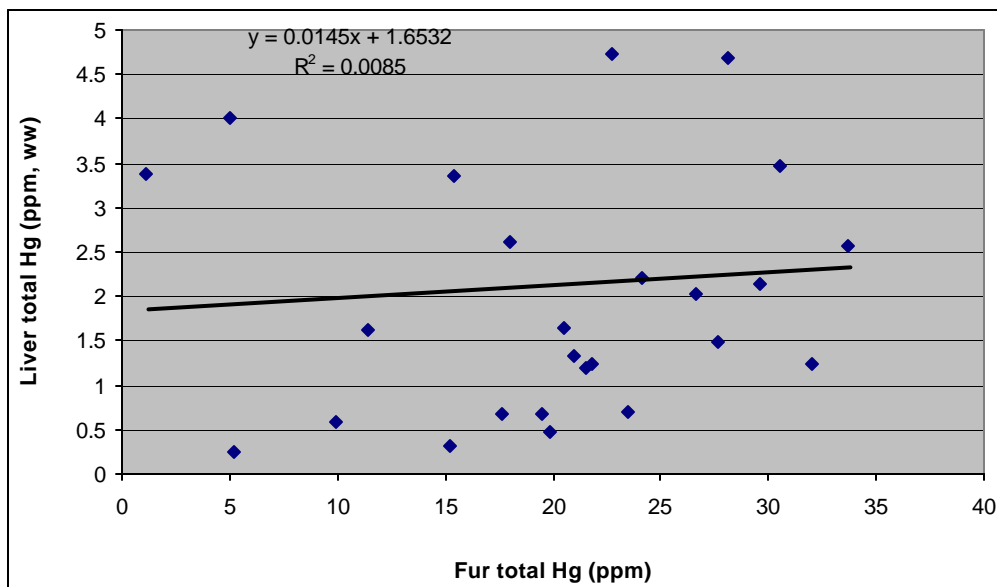
The relationship between fur and liver Hg levels were highly significant in mink ($r^2 = 0.60$, $F = 66.6$, $df = 45$, $p < 0.001$) (Figure 3a).

Figure 3a: Relationship between fur and liver Hg in Mink, Maine.



The relationship between fur and liver Hg levels were not significant in river otter ($r^2 = 0.009$, $F = 0.20$, $df = 23$, $p > 0.05$) (Figure 3b).

Figure 3b: Relationship between fur and liver Hg in River Otter, Maine.



D. Live Capture

Because few trappers operate in the Flagstaff and Seboomook regions we recommend further live trapping in these areas. Capturing a live animal permits blood sampling. Analysis of blood samples allow more meaningful comparisons among different sites and regions, because (1) blood Hg levels reflect a recent or short term Hg exposure of a piscivorous mammal and (2) should be independent of age. Because >95% of Hg in the blood is in the methyl form, measuring total Hg provides insight into the recent dietary uptake of MeHg. Collecting blood samples from recently killed animals is difficult because blood rapidly loses moisture after death; therefore, blood clots and whole blood Hg likely do not correlate (based on studies with loons). Conversely, much of the Hg in organs is inorganic. By sampling and analyzing fur and blood from live individuals we hope to establish a relationship between the two matrices that can be applied to future studies for Hg interpretation of live or dead animals. Because animals can be live-trapped in areas of low density, we avoid potential population impacts and provide a comparative template for other studies that cannot afford removing animals.

Live trapping also adds another matrix of Hg measurement that can be related to other compartments such as fur, liver, kidney, and brain. Each matrix provides different information. Mercury levels in fur are an indicator of long-term body burden and organs generally demethylate Hg and do not necessarily provide an accurate assessment on toxicity to the individual. There is now evidence that the brain can demethylate Hg (particularly in the otter, D. Evans pers. com.) so that compartment may not be helpful for chronic Hg loads. Sampling certain matrices, such as muscle or fur (since fur would likely reflect remobilization of MeHg in the muscle) can provide better insights into the lifetime body burden for the animal. This is crucial part of this investigation because the bioaccumulation rate of MeHg is one of the most important aspects of its toxicity to a population.

1. Efforts in 2001

BRI set a total of 28 leg-hold traps in the Flagstaff and Chain of Ponds area during September and October. The traps were set out for a total of 34 nights, resulting in the successful live-capture of one otter, on 05 October, as well as 5 other missed attempts in which otter were suspected (otter scat found near trap). We had a total of 952 trap nights (Table 5) and our success was 0.03 otters per night (Table 5). In a study by Blundell G.M. et al. (1999) the trapping efficiency for river otters using leg-hold traps was 0.048.

Table 5. Live trapping results for 2001.

Trap Function	Leg-Hold Traps
Efficiency (captures/trap night)	0.03
Trapping Effort (#traps*# of nights trapping)	952

The live-trapped otter was an adult male captured at Middle Chain of Ponds, located in Chain of Ponds Township, Maine. The otter was caught by his right rear foot. First, the otter was removed from the trap with the aid of a catchpole and placed into a catch box, enabling transportation back to the field station to take a blood and fur sample.

Once the animal was anesthetized we examined it for any obvious injuries that it may have sustained from the trapping process (no visible damage to the otter was observed).

A mixture of Ketamine 0.18 cc (2.5 mg/kg) and Metomidine 0.18 cc (0.025 mg/kg) sedative was administered via hand injection to the rump of the animal. Approximately three minutes following injection, the animal was fully sedated. The otter was removed from the catch box and placed upon a padded blanket where the sampling of tissue (blood and fur) and basic measurements (weight and length) were collected. A small patch of fur was clipped from the area located just above the animal's hind foot. Using a 1cc syringe, approximately 0.3cc of whole blood was hand-drawn from the jugular vein. The animal was then placed back into the catch box and was administered the 0.15 cc of the antiseden Atipamezole (0.10 mg/kg). Approximately 8 minutes proceeding the injection of Atipamezole was required for the otter to fully recover. The total time the otter was anesthetized was approximately 45 minutes.

The animal was kept overnight at the field station where it was monitored for any health irregularities and then was released the following morning at the trapping site.

2. Tissue Analysis

The otter whole blood and fur sample were analyzed for total Hg at Texas A&M University, College Station, Texas. The blood mercury level was 0.244 (ppm, ww) and the fur was 37.6 (ppm). Existing studies investigating levels of Hg within otter blood, which is needed in order to make a comparison with our live-trapped otter sample, have not been found. However, the total Hg value of 37.6 ppm found in the otter fur is significantly higher than the existing mean of 19.58 ppm (+/-8.60) in the 26 otter carcasses collected and analyzed from Maine in 2000-01 (Table 1).

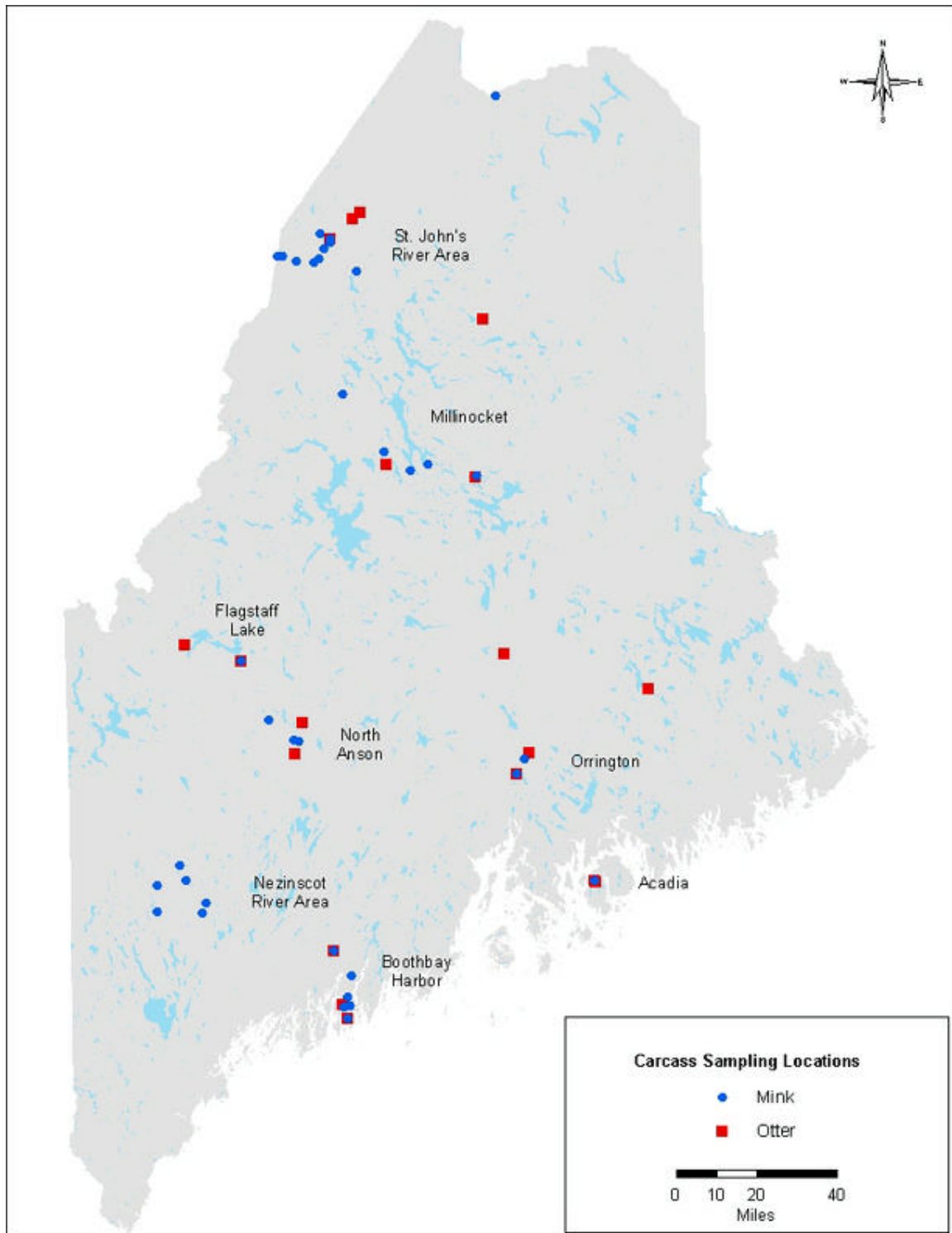
RECOMMENDATIONS

1. Continue carcass retrieval using our network of trappers from our three focal areas and add a new focal area in the Millinocket neighborhood;
2. Initiate live capture efforts in the Flagstaff Lake and potentially the Seboomook Lake areas to overcome the lack of trapped otter and mink in those areas;
3. Add a biomarker assay to provide insight on potential impacts from Hg with an emphasis on individuals with fur Hg levels over 20ppm.

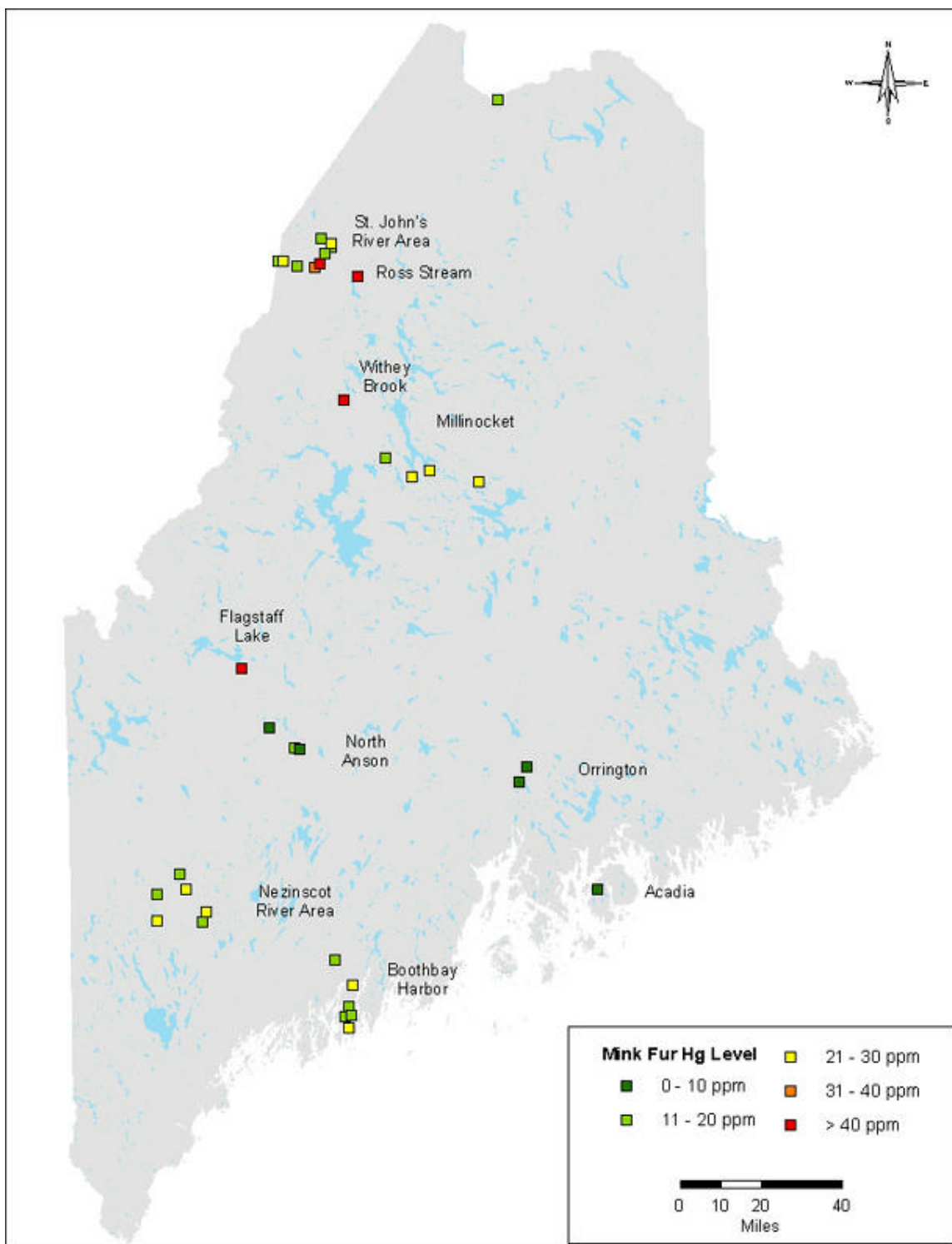
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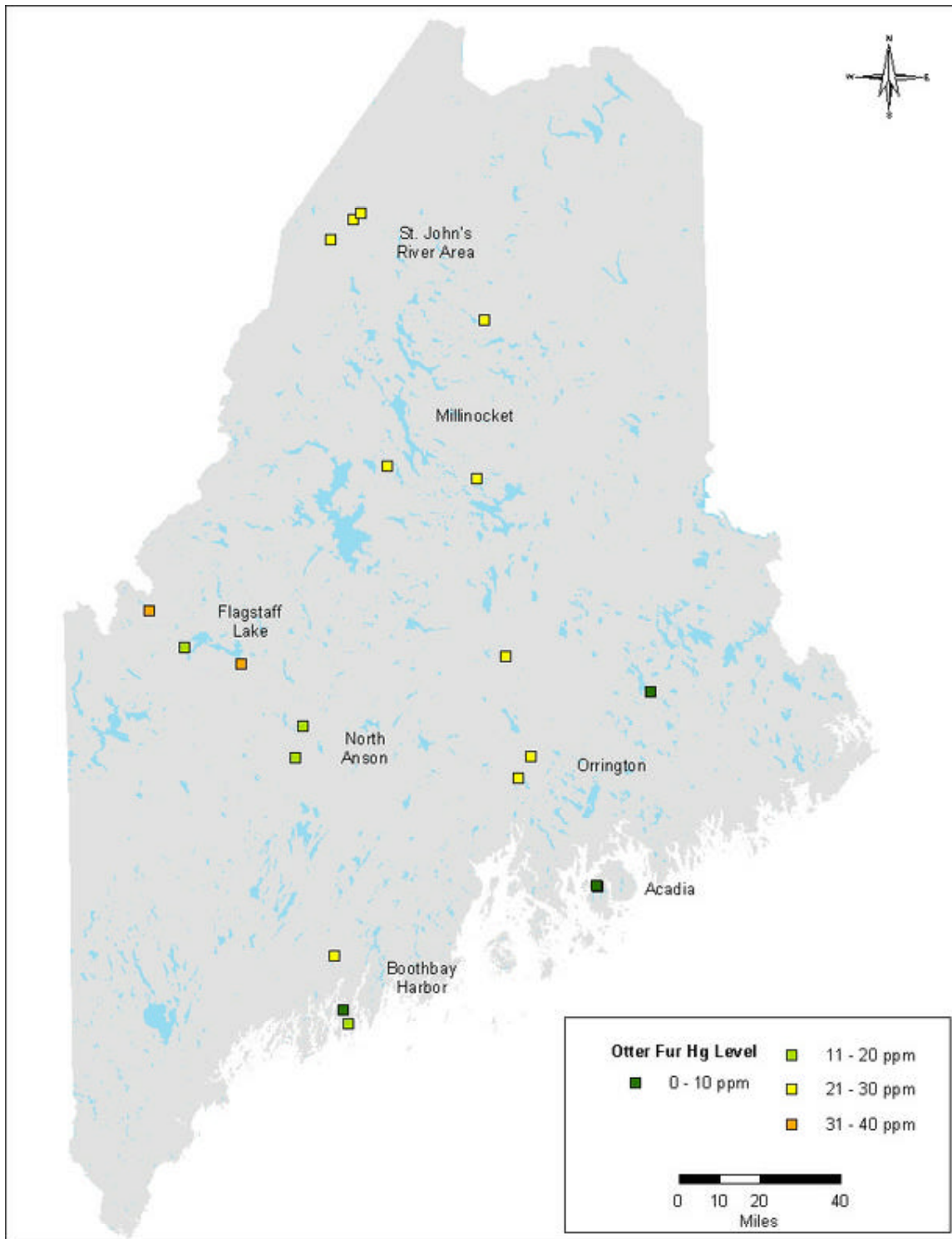
Appendix 1: Mink and River Otter carcass sampling locations, 2000-01.



Appendix 2: Mink carcass sampling locations, 2000-01.



Appendix 3: River Otter carcass sampling locations, 2000-01.



Appendix 4: Mink and River Otter live-trapping sites, 2001.

